Testing Guide for

GROWTH HORMONE (GH)

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1.0 Substance Class Overview

Growth hormone (GH) (also known as human growth hormone (hGH), somatotropin or GH1) is a naturally occurring peptide hormone which is secreted in a pulsatile pattern by the human pituitary gland at the base of the brain. GH circulates in blood in multiple molecular forms (i.e. isoforms of different masses e.g. 22 kDa, 20 kDa, 17.5 kDa) and their aggregated states (homo- and heterodimers of different isoforms). First isolated from humans in the 1950s, GH drives numerous physiologic processes, including skeletal and organ growth and maintenance, calcium homeostasis, lipolysis, and the regulation of metabolism and lean body mass. Daily secretion of GH increases throughout childhood, peaking during adolescence, and steadily declining thereafter. Recombinant hGH (rhGH) is composed of the monomeric 22 kDa GH (the main naturally occurring isoform), and it was developed and approved for specific clinical uses such as treatment of growth hormone deficiency in children and adolescents, pituitary insufficiency conditions and diseases that result in muscle wasting. In the case of an athlete receiving appropriate treatment with rhGH, an application for a therapeutic use exemption (TUE) should be completed.

An FAQ on GH can be found at: https://www.wada-ama.org/en/questions-answers/human-growth-hormone-hgh-testing

**TABLE 1: Reported WADA Testing Statistics - GH**

<table>
<thead>
<tr>
<th></th>
<th># of samples analyzed</th>
<th># of Sports</th>
<th># of TAs</th>
<th>AAFs¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018²</td>
<td>24,242</td>
<td>99</td>
<td>137</td>
<td>2</td>
</tr>
<tr>
<td>2017</td>
<td>20,482</td>
<td>90</td>
<td>124</td>
<td>0</td>
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<tr>
<td>2016</td>
<td>17,538</td>
<td>68</td>
<td>111</td>
<td>6</td>
</tr>
<tr>
<td>2015</td>
<td>13,264</td>
<td>74</td>
<td>103</td>
<td>4</td>
</tr>
</tbody>
</table>

2.0 Performance-Enhancing Benefits

Although controversy exists about its alleged benefits, GH is commonly abused by athletes and bodybuilders for its claimed ability to increase muscle mass and decrease body fat, as well as its purported potential to improve athletic performance. For a highly trained athlete, GH can help push performance further when combined with the use of anabolic steroids. It is also claimed that rhGH can shorten recovery times after high intensity workloads or injury. The extent to which GH can have an anabolic effect and the potential mechanisms mediating such effects at physiologic doses remain controversial.

In an interesting specific case in 2017, GH seems to have been used not for its effects on muscle growth but to aid recovery or act together to aid erythropoietic stimulation by CERA, as the two substances were injected at the same time. Co-treatment of GH and EPO or other agents affecting erythropoiesis has indeed been suggested to potentiate the EPO effects. However, the physiological effects of GH doping are generally only perceived when multiple doses are administered over at least a few weeks. The doping scenario presented, co-injection with CERA and GH the evening before the race, may indicate a misunderstanding of the kinetics of these drugs,

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¹ All AAFs in the period 2015-2018 are the result of analysis with the isoform test.
² Data to be confirmed once WADA 2018 Anti-Doping Testing Figures are published.
but the rise in confidence after taking a performance-enhancing drug has a known placebo effect that can also positively affect performance.

3.0 How Supplied

Commonly supplied in multi-dose vials containing pure white sterile powder that requires reconstitution with sterile diluent before injection. rhGH can also be obtained pre-mixed in auto-injector pen-like devices. Dosage may vary from 1 mg to 24 mg (0.3 – 8 IU) or more per vial or pre-loaded syringe. 1 International Unit (IU) = 3 mg. Distributed for licit and ilicit use in virtually all developed countries, also sold under the trade names Genotropin®, Humatrope®, Norditropin®, Nutropin®, Omnitrope®, Saizen®, Serostim®. The illicit distribution of injectable recombinant GH formulations is thought to be primarily through internet pharmacies, as well as wellness and anti-aging clinics and websites. Internet pharmacies are often partnered with a physician willing to write prescriptions for a fee without a physical examination. Individuals may also obtain GH without a prescription through the black market. GH is often marketed with other performance enhancing drugs (e.g., anabolic steroids). “Bioidentical” GH creams are also available but may not actually contain GH. Products are stored away from heat and may be refrigerated to increase shelf life.

4.0 Cost

Prices vary between moderate to expensive but can easily be obtained on the internet or black market providers. Athletes are known to ship via post/courier or transport products with them over international borders.

5.0 Routes of Administration and Dosing Regimes

Designed for subcutaneous (SC), intramuscular (IM) or intravenous (IV) injections. rhGH has a very short half-life, approximately 15-20 minutes. It has been demonstrated that serum concentrations of GH return to baseline approximately 8-16 hours after intramuscular injection and 11-20 hours after subcutaneous injection of rhGH. With a conversion rate of 3 IU/mg, an average hGH abuser may use as much as 6.67 times more hormone as a young adult, who receives a starting dose of 0.2 mg/d. When used for physique or performance-enhancing purposes, rhGH is usually administered at a dosage of 1 IU to 6 IU per day (2-4 IU being the most common) or 10–25 IU per day three-to-four times a week to increase lean body mass. The dosage for improving muscle mass, typically for bodybuilders, has been reported in the range of 3–8 mg/day, three to four times a week in cycles of four to six weeks. In clinical practice, the long-acting formulations are used once weekly (sometimes biweekly). In order to obtain benefits, rhGH has to be used for prolonged periods of time: it is commonly cycled in a similar fashion to anabolic steroids, with the length of intake generally being between 4 weeks and 24 weeks. Other drugs commonly used in conjunction with rhGH to elicit a stronger response include thyroid drugs, insulin, and anabolic steroids. In endurance sports, little is known about the optimal utilization of GH doping in combination with other products. Highly individual and anecdotal reports indicate both positive and negative effects on athletic performance.

6.0 Other Considerations

Although drug absorption is acceptable by both SC and IV injections, daily SC administration is generally regarded as the preferred method of using rhGH. Various oral preparations (e.g., sprays and pills) purported to contain GH are also marketed and distributed. However, GH is only bioavailable in the injectable forms since the GH molecule is too large for absorption across the lining of the oral mucosa and the hormone is enzymatically digested in the stomach before absorption can occur. Athletes may choose rhGH over other prohibited substances because they
know rhGH is not detectable in the urine and blood testing is much less frequent than urine testing, and because they know the half-life of GH in circulation is short, limiting its window of detection by direct methods. Insulin is also a commonly used PED for its purported anabolic properties such as stimulation of glycogen formation, which is important for muscle recovery after exercise.

There are a number of health risks of excess GH use, and may be consistent with acromegaly, a pathological syndrome characterized by bony overgrowth. Carpal tunnel syndrome, neuropsychiatric complaints, Impaired fasting glucose, diabetes mellitus, and various lipid disorders may develop as well as hypertension, cardiomyopathies, valve disorders, and arrhythmias.

7.0 Detection Matrices and Methods

There are two methods for GH detection, both of which are performed on serum and therefore require the collection of serum blood samples in specific tubes containing an inert polymeric serum separator and a clotting activation factor (SST II Vacutainer tubes).

a. GH Isoform Test

The GH isoform test was the first method of detection and is also called the GH isoform differential immunoassay. It exploits the fact that the administration of rhGH significantly alters the natural proportions of circulating GH isoforms in the blood. Altered isoform ratios are reported as adverse analytical findings when male- or female-specific decision limits are exceeded with both test kits employed for analysis. The ‘GH isoform test' was first applied for the Athens 2004 Olympic Games. Commercial kits, specifically developed for doping control purposes, are available from CMZAssay GmbH (Berlin, Germany) and have been applied for anti-doping testing since June 2008. A strength of this test is that it is aimed at the exogenous doping agent itself, although it cannot distinguish it from its endogenous counterpart. The major limitation of this differential isotope ratio test is its narrow window of detection (24-36 hr post administration). If a Sample is intended for GH analysis with the GH isoform test, it shall be analyzed within 96 hours from collection (for more details, please refer to TD2019GH).

b. GH Biomarkers Test

The GH biomarkers test is an indirect GH biomarker-based approach, which was initially applied during the London 2012 Olympic and Paralympic Games. The method was re-launched in 2015 since one of the assays that constituted the initial testing method was withdrawn from the market by the supplier and the re-validation of further assays was required. The ‘GH biomarkers test' is based on the quantification of two main markers of GH biological action whose concentrations in blood increase after the administration of GH, namely insulin-like growth factor-1 (IGF-1) and the N-terminal propeptide of type III procollagen (P-III-NP). Further, a score (known as the GH-2000 score) is calculated, which factors in the concentrations of IGF-1 and P-III-NP measured in the serum sample as well as the athlete’s gender and age. An adverse analytical finding is defined by a GH-2000 score exceeding the defined male- or female-specific decision limits applied to two different pairs of IGF-1 and P-III-NP assays employed for analysis. If a Sample is intended for GH analysis with the Biomarkers method, it shall be analyzed with 120 hours from collection (for more details, please refer to the Guidelines on hGH Biomarkers Test in effect).

c. Both the GH isoform test and the GH biomarker test are complementary as the GH isoform test is more sensitive to GH administration soon after use and has a detection window of approximately 24-48 hours, which depends on the level and frequency of dosage and the route
of administration, whereas the biomarkers approach has potential to detect sustained changes in IGF-1/P-III-NP levels with a detection window potentially of several days to several weeks, again subject to level and frequency of dosage and route of administration.

8.0 High Risk Sports

rhGH abuse has been reported in a wide variety of sports/disciplines, including strength (bodybuilding, powerlifting) and endurance (cycling, triathlon, athletics) or those requiring frequent and repetitive intense activity (sprinting, weightlifting, boxing) either during training or competition. rhGH may also be abused in multiple-stage races (e.g. between stages in multiple-day cycling events) and in tournament and competition settings with high physical demands over a period of time. For information on the minimum levels of analysis (MLAs) for GH refer to Appendix 1 and Appendix 2 of the Technical Document for Sport Specific Analysis (TDSSA).

IGF-1 doping

IGF-1 is a circulating biomarker and mediator of GH action so the marketing in 2005 of recombinant human IGF-1 for the treatment of diabetes, insulin or GH insensitivity or motor neuron disease, together with the availability of IGF-1 analogs, creates the possibility of IGF doping. The biological basis for ergogenic effects of IGFs is due to its GH-like effects, however this remains largely speculative and accompanied by the same safety concerns. IGF-1, IGF-2 and their analogs, as well as insulin and its analogs, are all readily detectable by LC-tandem MS. However, a specific test to directly detect recombinant IGF-1 doping remains to be established, however is detectable if IGF-1 has been modified such as in the case of long arginine 3-IGF-1, abbreviated as IGF-1 LR3 or LR3-IGF-1, is a synthetic protein and lengthened analogue of IGF-1.

Mechano-growth factor (MGF) is a name used for a splice variant of IGF-1 which, although not known to appear in the circulation, have any pharmacological effects or be approved for human use, is advertised on the black-market and internet for alleged anabolic or tissue repair/regeneration benefits. Like other short peptides with known structures, it is readily detectable using LC-tandem MS.

9.0 Suggested Testing Strategies

Detection of rhGH doping remains difficult, thus integration of analytic and non-analytical intelligence strategies is a key to success. Evidence discovered through tips and investigations is critical to selecting the right athletes and timing the testing to maximize chances of detection success.

rhGH can be abused at any point during the competition or off-seasons. Patterns of use may occur in areas where athletes believe testing is less likely or in hard to test locations for blood collections. It is recommended that the majority of testing for GH is done out-of-competition, since GH requires chronic use and as a guide a breakdown of 80% out-of-competition and 20% in-competition would be a good starting point. Manipulation of whereabouts is likely, thus monitoring travel patterns and whereabouts update frequency may be useful.

• Prioritize a list of sports/disciplines as outlined in the TDSSA and determine the level and number of athletes to be tested.
• Obtain non-analytical information about key events for the season, as well as team information regarding training and selection camps/events. Monitor whereabouts for abnormal patterns or patterns of whereabouts changes for training partners.

• Work backwards from key events and schedule testing unpredictably on all athletes, paying special attention to collecting blood at different times of the day and week, as well as samples at least two weeks before major events before athletes begin to taper their training.

• As natural GH levels spike soon after bed time, athletes may choose to take GH in the evening (also hoping that the drug will not be detected if a sample is collected the following day and tested with the GH isoform test), or after intense training.

• Early morning testing should be incorporated with other varied and unpredictable sample collection sessions.

• Follow-up on all suspicious laboratory results including atypical findings and other results showing elevated, but not adverse, GH ratios and scores and review supplementary report forms from sample collection sessions to act on any abnormal information. Request testing for Growth Hormone Releasing Factors (GHRFs), insulin and large peptide analysis (e.g. IGF-1 LR3) in urine samples from the suspected athletes.

• Monitor GH ratios and scores on the individual level over time and look for significant fluctuations.

• Because the “biomarkers test” and the “isoform test” have a different window of opportunity for detection, complementary use of both tests could be helpful to increase the likelihood of detecting GH. For the moment, ADOs could focus their resources on the GH isoform analytical method, which is available in all WADA accredited laboratories and has resulted in the majority of AAFs. In specific instances, consider targeted tests requesting both detection methods to close potential gaps in detection because of rhGH administration timing.

• Combining samples together (batching) for the same analytical method is often a more cost-effective option. However, this is subject to the laboratory’s capacity and the timing requirements for reporting of results.

• Store GH samples for future analysis and/or re-analysis when further technological advancements for GH analysis are available.

• Consider interviewing any athletes with past adverse analytical findings to learn about dosing and use patterns and monitor athlete discussion boards to learn about evolution in GH abuse.
10.0 References

WADA 2017 Anti-Doping Testing Figures

WADA 2016 Anti-Doping Testing Figures

The Technical Document TD2019 GH - Human Growth Hormone (hGH) Isoform Differential Immunoassays For Doping Control Analyses


